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23416 7590 07/21/2010 CONNOLLY BOVE LODGE & HUTZ, LLP P O BOX 2207 WILMINGTON, DE 19899			EXAMINER WORLEY, CATHY KINGDON	
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**BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES**

Application Number: 10/593,181
Filing Date: September 15, 2006
Appellant(s): HILLEBRAND ET AL.

Roberte M. D. Makowski, Ph.D.
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed May 31, 2010, appealing from the Office action mailed Dec. 2, 2009.

(1) Real Party in Interest

The examiner has no comment on the statement, or lack of statement, identifying by name the real party in interest in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The following is a list of claims that are rejected and pending in the application:

Claims 1-30 are pending.

Claims 5-9, 11-26, and 28-30 are withdrawn for being directed to non-elected inventions.

Claims 1-4, 10, and 27 are rejected.

(4) Status of Amendments After Final

The examiner has no comment on the appellant's statement of the status of amendments after final rejection contained in the brief.

(5) Summary of Claimed Subject Matter

The examiner has no comment on the summary of claimed subject matter contained in the brief.

(6) Grounds of Rejection to be Reviewed on Appeal

The examiner has no comment on the appellant's statement of the grounds of rejection to be reviewed on appeal. Every ground of rejection set forth in the Office action from which the appeal is taken (as modified by any advisory actions) is being maintained by the examiner except for the grounds of rejection (if any) listed under the subheading "WITHDRAWN REJECTIONS." New grounds of rejection (if any) are provided under the subheading "NEW GROUNDS OF REJECTION."

WITHDRAWN REJECTIONS

The following grounds of rejection are not presented for review on appeal because they have been withdrawn by the examiner. The rejection of claims 1-4, 10, and 27 under 35 USC 112, first paragraph for a lack of written description has been withdrawn in light of the Applicant's amendments to the claims that were submitted on Mar. 8, 2010.

(7) Claims Appendix

The examiner has no comment on the copy of the appealed claims contained in the Appendix to the appellant's brief.

(8) Evidence Relied Upon

Signer et al (WO 01/96583; published on Dec. 20, 2001) – EXHIBIT A

Nasholm et al (WO 03/060133; published On July 24, 2003) – EXHIBIT B

Stougaard, J. (The Plant Journal (1993) Vol. 3; pp. 755-761) – EXHIBIT C

Boeke et al (Methods in Enzym. (1987) Vol. 154; pp 164-175) - EXHIBIT D

Hare et al (Nature Biotech (2002) Vol. 20; pp. 575-580) – EXHIBIT E

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claims 1-4, 10, and 27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Signer et al (WO 01/96583; published on Dec. 20, 2001) in view of Nasholm et al (WO 03/060133; published On July 24, 2003) and taken with the evidence of Stougaard, J. (The Plant Journal (1993) Vol. 3; pp. 755-761) and the evidence of Boeke et al (Methods in Enzymology (1987) Vol. 154; pp. 164-175).

The claims are directed to a method for producing a transgenic plant by transforming a plant cell with a nucleic acid encoding a D-amino oxidase and with a second nucleic acid conferring an agronomically valuable trait, and utilizing D-amino acids for both a positive selection step and a negative selection step, followed with breaking the combination between the two nucleic acids.

Signer et al teach a method of generating transgenic plants that utilize both a positive selection marker and a negative selection marker in order to remove the selection markers from the resulting transgenic plants (see entire document). They outline a general protocol on page 11:

- 1) providing a DNA construct which comprises (a) direct repeats of a gene of interest at both ends flanking a positive selectable marker gene and a negative selectable marker gene and (b) one or more additional genes that flank either side or both sides of (a);
- 2) transforming cells by introducing the construct into the cells;
- 3) growing or culturing the cells on positive selective medium;
- 4) selecting the transformed cells having the genetic construct which grows on the positive selective medium;
- 5) transferring the cells to a negative selective medium;
- 6) growing or culturing the cells on the negative selective medium;

And

- 7) selecting those cells which grow on the negative selective medium.

Growth on the negative selective medium indicates that the selection markers have been excised.

Signer et al do not teach a sequence encoding a D-amino acid oxidase gene for use as either a positive or a negative selection marker; therefore, their construct is different from the construct recited in the instant claims.

Nasholm et al teach that D-amino acids may be used for selection of transgenic plants expressing a D-amino acid metabolizing protein (see page 3). They teach that the D-amino acid metabolizing protein can be a D-amino acid oxidase (see line 21 on page 5). They teach that D-amino acid oxidase could be used as a positive selection marker with D-alanine and D-serine because D-amino acid oxidase would alleviate the toxicity caused by D-alanine and D-serine (see fourth paragraph on page 35). They also teach that D-amino

acid oxidase could be used as a negative selection marker with D-isoleucine because applying D-isoleucine to plants expressing D-amino acid oxidase hindered the growth of the transgenic plant with no visible inhibitory effect on wild type plants (see first paragraph on page 36).

At the time the invention was made, it would have been obvious and within the scope of one of ordinary skill in the art to modify the teachings of Signer et al to utilize a construct encoding a D-amino acid oxidase as taught by Nasholm et al. One would have been motivated to do so, because Nasholm et al taught that one transgene (encoding D-amino acid oxidase) could be useful as both a positive and a negative selection marker, and therefore one would only require one transgene rather than two separate selectable marker genes.

This concept was generally known in the art as evidenced by the teachings of Stougaard (see entire article) and Boeke et al (see entire article). Stougaard teaches transgenic tobacco expressing a transgene encoding CodA which can be used for positive selection on N-(phosphonacetyl)-L-aspartate containing medium and can be used for negative selection on 5-fluorocytosine containing medium (see abstract). Boeke et al teach that the URA3 gene can be used in yeast as both a positive selectable marker and as a negative selectable marker (see figure 1 on page 166). It is common practice in yeast genetics to use a plasmid comprising the URA3 gene as a positive selectable marker by growing URA3- yeast on medium lacking uracil, therefore only the transformants carrying the URA3 gene are able to grow (positive selection). For a negative selection, the yeast

would be plated on medium containing 5-FOA (5-fluoroorotic acid) (see Figure 1 on page 166).

Given the effect of different D-amino acids on transgenic plants expressing D-amino acid oxidase as taught by Nasholm et al, and given the success in using both a positive selection and negative selection to identify transgenic plants and subsequently identify plants in which the marker has been excised that was taught by Signer et al, one would have had a reasonable expectation of success in combining the teaching to arrive at a method that utilizes D-amino acid oxidase as both a positive and negative selectable marker, as claimed in the instant application.

(10) Response to Argument

Most of the Applicant's arguments are an attack on the references individually rather than considering them as a whole in combination. In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

One example of the Applicant's attack on the references individually is the argument presented on pages 8-10; that Signer teaches two different selectable markers instead of a single dual function marker, that Stougaard teaches CodA, but continues to utilize two distinct markers, and the Boeke teaches selection in a medium lacking uracil instead of selection in medium that is toxic to wild-type cells. This is an attack on the references

individually, because Boeke teaches the use of one dual function selectable marker (the URA3 gene), and Nasholm et al teach that amino acid oxidase can be used as both a positive and a negative selectable marker. Therefore, these limitations are covered in the combination of references. The fact that the URA3 gene is used as a positive selection based on auxotrophy (the inability to grow on media that lack an essential component – in this case uracil) instead of being used as a positive selection based on media comprising a toxic compound, is not a relevant fact; because Nasholm et al teach D-amino acid oxidase and the compounds "X" (ie. D-Serine, D-Alanine) and "M" (ie. D-isoleucine).

Another example of the Applicant's attack on the references individually is the argument presented on pages 10-16; that Signer's construct is different than the claimed construct because it utilizes direct repeats of the gene of interest and it utilizes two different selectable markers, and that Boeke does not teach positive and negative selection using two selection media that act on one selectable marker. This is an attack on the references individually because Nasholm et al teach the construct of the instant claims, and Nasholm et al teach the different media that can be used for positive selection (containing D-alanine and D-serine - see fourth paragraph on page 35) or negative selection (containing D-isoleucine – see page 36).

Another example of the Applicant's attack on the references individually is the argument presented on pages 16-17; that substituting the D-amino acid oxidase gene taught by Nasholm for the CodA gene taught by Signer would result in a construct comprising the NPT gene and the D-amino acid oxidase gene, rather than comprising D-amino acid oxidase alone. This is ignoring the teachings of Boeke et al showing that one selectable

marker can be used for both positive and negative selection, and therefore the D-amino acid oxidase would replace both the NPT gene and the CodA gene.

Another example of the Applicant's attack on the references individually is the argument presented on pages 17-19; that none of the references point to using D-amino acids as claimed. This is ignoring the teachings of Nasholm et al, that D-amino acids can be used as either positive selection or negative selection with D-amino acid oxidase as a selectable marker; and it is ignoring the teachings of Signer et al, who teach that one can use positive selection, first, to identify transformants, and follow up with negative selection to select for transformants that no longer carry the selectable marker gene.

The Applicant specifically argues that dependent claim 10 would not have been obvious for the reason that the second expression cassette is not localized between the sequences which allow for specific deletion of the first expression cassette (see pages 19-20). This is not persuasive, however, because Stougaard teaches a gene of interest, hph for pNE7 and AcTpase for pNE5, that is adjacent to the selectable marker gene (see Figure 2 on page 756). Therefore, the limitation that the second expression cassette is not localized between the sequences which allow for specific deletion of the first expression cassette is taught by Stougaard. Furthermore, it was generally known at the time of filing that there are different approaches for excising selectable markers (see review by Hare et al (2002) Nature Biotechnology; Vol. 20, pp. 575-580); such as: homologous recombination (as taught by Signer), recombination induced by recombinases (ie. the Cre/Lox system) (see page 577 of Hare et al), mobilization of transposons (see page 577 of Hare et al), or segregation of plasmids that have been transformed into the transgenic plants separately (see right column

on page 576 of Hare et al). Given the general knowledge in the art at the time of filing, including the second expression cassette outside of any sequences that will allow for deletion of the selectable marker is an obvious design choice. The fact that Signer et al taught a more complex system, in which the gene of interest was interrupted by the selectable marker such that it would be restored upon the excision of the selectable marker, does not render non-obvious a more simple system of deleting the selectable marker which is adjacent to the gene of interest.

The Applicant specifically argues that claims 3 and 27 specify a step of inducing deletion of the first expression cassette, and the method of Signer does not induce deletion of the selectable markers but rather waits for the recombination to occur naturally (see page 20). This is not persuasive, however, because claims 3 and 27 do not recite any active method steps or materials that are utilized for "inducing deletion of said first expression cassette" (see part "v" of claim 3). Therefore, the method of Signer et al, in which the plants are cultured until recombination occurs naturally, satisfies the limitation of "inducing" deletion, because the act of culturing the plants and allowing sufficient time and growth is what is required for "inducing" the recombination events taught by Signer et al. Furthermore, as taught in Hare et al, many different methods for inducing deletion of selectable markers were known in the art at the time of filing.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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